Senova Von Willebrand Factor Propeptide ELISA Test Kit (ELISA VWF:PP)

Solid phase immunoassay (ELISA) for Von-Willebrand-Factor Propeptide (VWF:PP) in human blood plasma

In-Vitro Diagnostic Product
Not For Therapeutic Use
For Professional Use Only

CE IVD

REF **2.7.2.27**

Intended Use

ELISA VWF:PP is an enzyme immunoassay for the quantitative measurement of von-Willebrand-factor-propeptide (VWF:PP) in human blood plasma.

Diagnostic Importance

Von Willebrand factor (VWF) has several important functions in primary haemostasis. The multimeric protein is found in plasma, in thrombocytes and endothelial cells. VWF is also a carrier protein and stabilizer for coagulation factor VIII (FVIII) in plasma. During the biosynthesis of VWF multimers a 100 kDa glycoprotein propeptide (VWF:PP) is proteolytically cleaved and released in plasma. In certain types of VWF disease, including hereditary or acquired forms, mutations and in a few other diseases, abnormal levels of both VWF:PP and VWF:AG or VWF activity can be found, highlighted in the ratio of VWF:PP and VWF:AG. Patients with VWF disease show an increased bleeding tendency, for example epistaxis, gum bleeding, hematoma, or excessive bleeding after dental or other surgical interventions. In in extreme cases of absolute deficiency (type 3 VWF disease), life threatening bleeding may occur.
In blood, VWF and VWF:PP show very different half-lives (12 versus 2 hours) (1). Measurement of VWF:PP is an important tool along with VWF:AG in characterizing the type of VWF deficiency, especially in patients with a shortened plasma half-live of VWF. (2). Further information can be found in the literature that is referenced at the end of this instruction sheet.

Principle of the Assay

The wells of the plastic strips included in this kit are coated with a monoclonal antibody directed against VWF:PP. The sample is pipetted into a well, followed by a second monoclonal antibody against VWF:PP that is conjugated with an enzyme. VWF:PP binds to the antibody attached to the solid phase and is immobilized. The second antibody with the conjugated enzyme binds to the immobilized VWF:PP as well. After incubation and washing steps all unbound material is removed and substrate added is cleaved by the bound enzyme of the conjugate and releases a dye in proportion to the bound VWF:PP. The reaction is stopped after a set time using a stop solution and the absorbance measured. This indicates the concentration of VWF:PP. The assay is calibrated by parallel measurement of the included calibrator and its dilutions via a calibration curve. A quality control is possible by simultaneous analysis of the control plasma, which is included in the kit.

Content of the Kit

The content of this kit is sufficient for 96 measurements:

12 **Micro-strips**: Strips with 8 wells, coated, individually shrink-wrapped with desiccant bag

1 Plastic **frame** for holding up to 12 strips for processing the assay

1 Bottle of **Sample diluent**, 25 ml, a buffer for pre-dilution of the sample

2 Bottles of **washing buffer,** 25 ml each, 10x concentrated, for dilution with water

2 Vials of **detector conjugate**, 3 ml each

2 Vials of **substrate solution**, 6 ml each, for detection

1 Vial of **stop solution**, 15 ml, for terminating the reaction

1 Vial of **lyophilized calibration plasma** for reconstitution with 1 ml water

1 Vial of lyophilized **control plasma** for reconstitution with 1 ml water

1 box insert / instructions for use

1 sheet with values for calibrator and control

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Required Material not included in the Kit

Distilled water, or preferably sterile water for injection

Calibrated pipettes and pipette tips

Equipment for blood sampling, if the venipuncture is made directly by the operator

Microplate reader with 450 nm and 620 nm as reference wavelengths

Multichannel pipette and reservoir for wash buffer, or automated washing device

Blotting paper or other absorptive stock material for wash solutions

Plastic tubes for preparation of dilutions or storage of samples

Stop watch

Shaking device for incubation

Storage and Stability

The product should be stored at +2 to +8°C / +35 to +46 °F. Under these conditions, it may be used until the expiration date printed on the label. Do not use after this date. Do not freeze any content of the kit.

This product may be shipped at ambient temperature if the temperature during transportation does not rise above +25 °C / +77 °F. It should be immediately stored at +4°C / +39 °F on receipt. Store all components of the kit inside the box provided, in order to protect from sun light.

Stability after opening:

Use the shrink-wrapped strips immediately after opening. Discard them after use, also if unused wells are left. Store all liquids at +2 to +8 °C / +35 to +46 °F after opening. Sample diluent, washing buffer and stop solution may be used up to 6 months after opening. Detector conjugate and substrate solution are stable for 2 months after opening. Calibrator and control may be used for up to 8 hours after opening. It is recommended to store them frozen in aliquots. The reconstituted and frozen calibrators and aliquots are stable for 6 months when stored at -18 °C / 0 °F or lower.

Recommendations and Precautions

This product is designed for use by qualified staff for diagnostic (EU) or research purposes only.

The results obtained from use of this kit should only be used as an additional information and an adjunct to other available diagnostical procedures and information to the physician.

Before use of this kit, carefully read the instructions for use and follow it strictly.

Do not use any components of this kit for other assays, or components of other kits for this assay.

Do not use any liquids that are turbid or contain particulate matter. On cooling, components of the 10-fold concentrated wash buffer may precipitate. These can be dissolved by bringing the solution to room temperature under swiveling or stirring and swirling the container. If particles remain, the buffer cannot be used. Do not take aliquots from this buffer as long as there are turbid or visible undissolved particles.

The freeze-dried calibrators and controls contain human plasma, which has been tested for the absence of HBV, HCV, HIV and Treponema pallidum. However, no assay can guarantee the total absence of infectious agents. Therefore, any product of human origin must be handled with all the required precautions, as if being potentially infectious.

The concentration of the material in the vials is specific to each batch of the kit individually, in order to optimise performance characteristics.

Calibrator and control contain very low concentrations of a broad-spectrum biocide as a preservative. Except the stop solution, the reconstituted or ready-to-use solutions are non-hazardous materials. Nevertheless, contact with skin or mucosa should be avoided. Hazard notice concerning the stop solution is provided at the end of the instructions for use.

Do not ingest any of the solutions!

Dispose any components after the test or residual material according to your national or laboratory guidelines. Unused product, residual material and used products, the contents of the micro strips should be disposed as clinical waste. The desiccant bags and the pouches of the micro strips may be disposed with the regular waste.

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Sample Preparation

The assay requires citrated plasma samples. Preferably, draw whole blood into a collection device for citrate anti-coagulated blood by venipuncture. Alternatively, nine parts of freshly drawn whole blood are mixed with one part of 0.11 mol/l sodium citrate solution. Test the sample within one to two hours after sampling and no later than eight hours.

For preparation of platelet poor plasma, a validated laboratory method should be employed, for example centrifugation at room temperature for 15 minutes at 2000 g. The plasma obtained by this procedure should be used within one to two hours; do not exceed 8 hours. Discard older samples. The plasma samples may be frozen.
The sample is stable at -20 °C / -4 °F for two months. Thaw in a water bath at +37 °C / +98 °F.

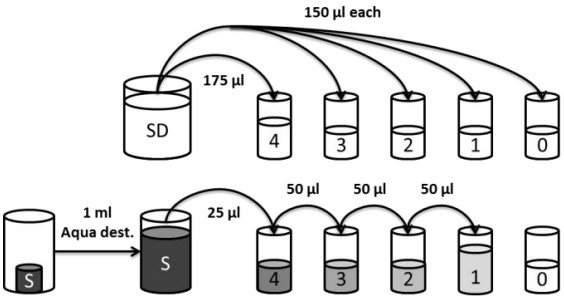
Preparation of Reagents

Make sure all the required reagents are taken from the same kit. Do not mix components of different batches of kits. Bring all reagents to room temperature prior to use.
Calibrator and control are freeze-dried and need to be reconstituted prior to use. Vials are under vacuum; remove the stopper after opening of the screw cap carefully in order to avoid any loss of material. Do not mix up the stoppers of calibrator and control. Add exactly 1 ml of distilled water free of preservatives. Close the vial with its screw cap and dissolve the lyophilized material by gentle agitation or with an orbital shaker. Avoid harsh shaking and foam formation. After reconstitution, the solutions should stand for 20 minutes prior to use, with gentle agitation from time to time and especially immediately before use. Additional mixing is not required during usage.
Add the required volume of wash buffer concentrate in a plastic tube of appropriate size and dilute it with 9-fold volume of distilled water. Mix well. For one well 4 x 0.3 ml = 1.2 ml of diluted wash buffer are required, or for one strip = 8 wells = 9.6ml. All other components are ready to use.

Test Procedure

Preparations:
Dilute the plasma samples according to the expected concentrations of VWF:PP exclusively with the sample diluent included in the kit. If a regular concentration is expected, a dilution of 1:80 is recommended. If a concentration below the range (<300 mIU/ml) is expected, a dilution of 1:20 is appropriate. Dilute control identically to sample. The control represents the regular range of the VWF:PP in plasma. A confidence range for each batch is indicated on the vial and in the separate sheet in the kit. The indicated concentration represents the value in the reconstituted control prior to dilution. It is recommended that each lab should determine its own confidence ranges.

Preparation of the standard curve:
Using 5 small tubes (No. 0-4). Dispense 175 µl sample diluent (SD) in tube 4, and 150 µl respectively into tube 0-3. Dispense 25 µl reconstituted calibrator (S) into tube 4 and mix carefully, but avoid foam formation. Dispense 50 µl from tube 4 into tube 3 and mix. Repeat these steps for tube 2 and 1. See the following scheme example below.



This procedure provides a dilution series with four (4) different standard concentrations for establishing a calibration curve (tubes 1-4) and a blank value (tube 0). If more calibration points are needed, or for different concentration ranges, you may generate additional dilutions. However, it is recommended to follow the proposed dilution scheme when using this product for the first time, and only establish your own dilution scheme with more experience later on.

The concentration of VWF:PP as indicated on the vial and the value sheet of the calibrator is found in tube 4. The other tubes contain the respective concentrations obtained by the dilution steps.

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Assay:

1 Calculate the number of wells required and open the respective number of pouches. Each pouch contains one strip with 8 wells. Insert the strip into the provided frame.

2 Dispense 50 µl respectively of a sample, blank, calibrator and control prepared as described above into separate wells.

3 Dispense 50 µl of the detector conjugate into each well immediately.

4 Incubate the samples for 120 min at room temperature on orbital shaker at 300 min^{-1}. Alternatively incubate for 60 min at +37 °C / +98 °F, also with shaking at 300 min^{-1}.

5 Remove the liquid by inverting the frame over blotting paper or other absorptive material and tapping.

6 Wash the wells 4 times with 300 µl wash buffer. Empty each well after each washing step.

7 After the last washing step, empty the wells as described in step 5 by tapping on absorptive material.

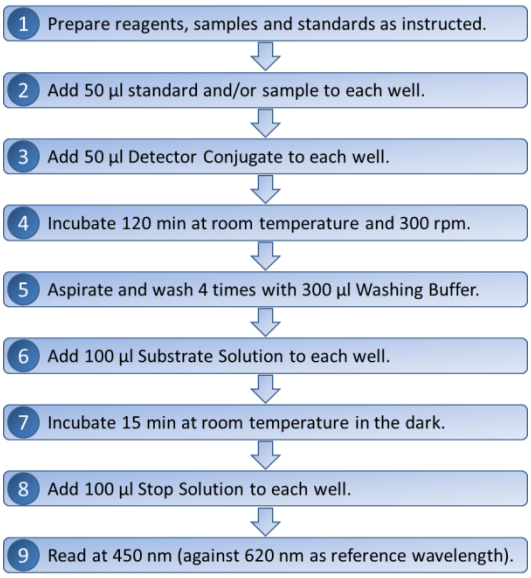
8 Dispense 100 µl of substrate into each well.

9 Incubate in the dark at room temperature for 15 minutes, (for example with aluminum foil covering).

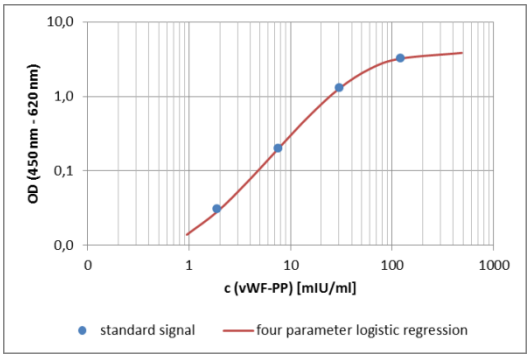
10 Dispense 100 µl stop solution into each well.

11 Immediately measure the absorbance at 450 nm wavelength against a reference wavelength of 620nm. The generated color is stable for about 30 minutes at room temperature. All wells need to be measured during this time.

Overview on all steps for illustration:



Calibration:
Prepare a calibration curve for each test with four dilutions as described above, by plotting the difference of the measured optical density (OD 420 - 620 nm) against the concentration in mIU/ml.
Generate a 4-point logistic fit or different appropriate regression method. An example is shown in the following figure:



To calculate the concentration of VWF:PP, multiply the sample by the dilution factor used. For any new assay, a fresh calibration curve is required.

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Quality Control

Calibration and sample results need to be verified by comparison to the provided control. Results in the samples should only be used if the control is found in the assigned confidence range for the control.
This control is prepared from pooled plasma of several donors with VWF:PP values in the normal range. Each lot of control and calibrator is assayed against the SSC/ISTH secondary coagulation standard lot#4.

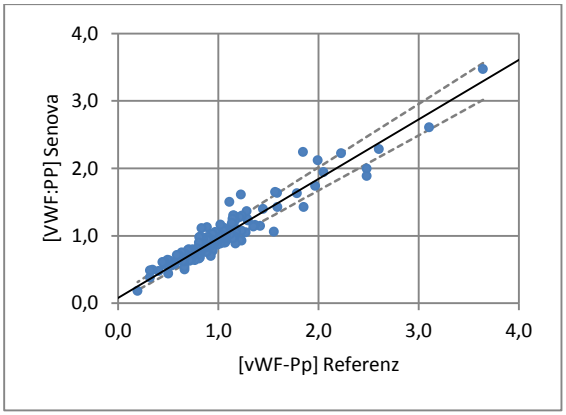
Expected Values

The published range of VWF:PP is in the range of 550-2190 mIU/ml without considering blood groups (6). However, each lab should determine its own normal range. It cannot be excluded that results of subject with blood group 0 will vary from those with blood group A, B, or AB.

Assay Characteristics

The following data was obtained on an Infinite PRO microplate reader (Tecan, Austria GmbH).

Detection threshold	2.5 mIU/ml
Lowest detectable concentration	5 mIU/ml
Measuring range	2.5 – 120mIU/ml



Method comparison ELISA Senova vs reference method

Method comparison:
162 samples from ostensibly healthy subjects and patients with various subtypes of VWF deficiency were compared with a different ELISA for VWF:PP.
A correlation coefficient of 0.9037 was found (Wellhöfer et al. Hämosteseologie 2019). The solid line in the diagram represents the regression after Passing and Bablok, the hatched line the minim and maxim values for slope and intercept with the y-axis.

Intra-assay precision:
Three samples prepared from pooled plasma with variable known concentrations of VWF:PP obtained by dilution were measured 8-fold in one run.

CV at 60.6 mIU/ml	0.1%
CV at 15.2 mIU/ml	3.4%
CV at 3.8 mIU/ml	8.1 %

Inter-assay-precision:
Three samples of known concentration of VWF:PP determined by ELISA were measured on eight different lots of this assay. Different concentrations of VWF:PP were obtained by dilution.

CV at 60.6 mIU/mL:	0.6 %
CV at 15.2 mIU/mL:	3.4 %
CV at 3.8 mIU/mL:	9.8 %

Day-to-day-precision:
Three different operators used one identical lot of this assay at four days following one another, respectively, to determine the concentration of Control N.

CV at dilution 1:20:	7.8 %
CV at dilution 1:80:	5.5 %

Operator-to-Operator Precision:
Three different operators tested an identical lot of this assay with the identical sample of Control N simultanously.

CV at dilution 1:20:	4.9 %
CV at dilution 1:80:	2.8 %

Specificity and Interferences:
This assay was developed for the specific and precise measurement of VWF:PP in plasma. The results did not show any influence by the plasma concentration of VWF. By using a specific reagent, interference by heterophilic antibodies or rheumatoid factors is very unlikely.

Haemolytic samples:
It is recommended to discard hemolytic samples; nevertheless weekly hemolytic samples could be tested successfully.
Icteric samples
Samples with a bilirubin concentration up to 80 µg/mL may be used for this assay.
Lipemic samples
Samples with a triglyceride concentration up to 3.5 mg/mL may be used for this assay.

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Limitations

Results obtained with this assay should not be used as the solid basis of a clinical diagnosis or for therapy. VWF:PP is primarily used to characterize the subtypes of VWF deficiency that show a shortened plasma half-life (1,2,3,4), but also in the differentiation of congenital and acquired forms of VWF deficiency (5). For this purpose the concentration of VWF:AG is measured with a suitable method and the ratio of VWF:PP and VWF:AG is calculated. Stufano et al (5) report a reference range of 0.6 to 1.6, and for differentiation of normal and patients with Vicenzy type mutation a cut-off value of 2.0. For differentiation of normals, patients with normal or mildly abnormal clearance of VWF or patients with acquired VWF deficiency, Stufano et al propose a cut-off value of 3.9. Since method specific details in the determination of both need to be considered and the case numbers were low, each lab should determine its own reference values.
Abnormal results of VWF:PP or the ratio of VWF:PP/ VWF:AG were found by vascular disorders like TTP (thrombotic thrombocytopenic purpura), in sepsis, in diabetes and other diseases also (1).

Hazard Warning

Stop solution:

Warning

Stop solution
H290: Can be corrosive to metals.
P234: Store in original packaging only.

Symbols

- Manufacturer
- CE-marked in accordance with IVD guideline 98/79/EG
- Number of tests
- Storage temperature
- Follow the instructions for use
- Expiration date
- For single use only
- IVD in vitro-diagnostic product
- LOT Lot number
- REF Reference number

Rev. Date 2020-01-24
No. 1.3.5.1765-1

Literature

1 Haberichter SL.VWF propeptide in defining VWD subtypes. Blood. 2015;125(19):2882-3

2 Haberichter SL. von Willebrand factor propeptide: biology and clinical utility. Blood. 2015;126(15):1753-61

3 Ruggeri ZM. Von Willebrand factor, platelets and endothelial cell interactions. J Thromb Haemost 2003;1(07):1335–1342

4 Borchiellini A, Fijnvandraat K, ten Cate JW, et al. Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. Blood 1996;88:2951–2958

5 Stufano F, Boscarino M, Bucciarelli P, Baronciani L, Maino A, Cozzi G, Peyvandi F. Evaluation of the Utility of von Willebrand Factor Propeptide in the Differential Diagnosis of von Willebrand Disease and Acquired von Willebrand Syndrome. Semin Thromb Hemost. 2019;45:36-42.

6 Haberichter SL, Balistreni M, Christopherson P, Morateck P, Gavazova S, Bellissimo DB, Manco-Johnson MJ, Cox-Gill J, Montgomery R. Assay of the von Willebrand Factor (VWF) Propeptide to identify patients with type 1 von Willebrand disease with decreased VWF survival. Blood. 2006;108(10):3344-3351

Additional literature is available through the manufacturer on request.

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